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Fractionation of triticale, wheat, barley, oats, canola, and mustard straws for the production of carbohydrates and lignins

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ABSTRACT

Five cereal (triticale, durum wheat, CPS wheat, feed barley, oats) and two oilseed (canola, mustard) straws were fractionated with pressurized low polarity water in a flow-through reactor at 165 °C with a flow rate of 115 mL/min and a solvent-to-solid ratio of 60 mL/g. The conversion and extraction of the major carbohydrates and lignin from the reactor system during hydrothermal treatment was largely completed within the first 20–30 min. Glucan content of all straws were enriched by the process. More than 90% of the xylan and nearly 50% of the lignin were extracted and there was no effect on yield due to crop species. However, there were differences in solid residue and liquid extract composition. Cereal crops yielded a residue richer in glucan and lower in lignin. Oilseed crop residues contained very low levels of ash. Xylo-oligosaccharides from oilseed crops contain more acetyl and uronic acid substituents.

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1. Introduction

Lignocellulosic biomass is one of the most abundant materials in the world and its production potential is approximately 200 billion metric tons annually (Zhang et al., 2007). There has been a lot of attention given to the utilization of this abundant and sustainable resource for use as the raw material for the production of energy and chemical needs (Kamm and Kamm, 2004; Ragauskas et al., 2006; Zhang, 2008).

Sources of lignocellulosic biomass include municipal waste, agricultural residues, hardwood and softwood, and dedicated biomass crops. It is estimated that there is 1.5 billion metric tons of dry lignocellulosic biomass from the residue of seven of the major agricultural crops (corn, wheat, barley, oats, rice, sorghum, and sugar cane) grown world-wide each year (Kim and Dale, 2004).

In Canada wheat, barley, and oats are the major cereal crops grown on the prairies. Total straw production from these crops is estimated to be 37 million metric tons, of which, 15 million metric tons is considered available for industrial use after soil and livestock requirements are met (Sokhansanj et al., 2006). There has been an increased interest in the use of another cereal crop, triticale, as an industrial crop for the production of energy and chemicals in a biorefinery system. Triticale, a hybrid of wheat and rye, yields more grain and produces more biomass (straw) than other cereal crops (Salmon et al., 2004). As such, worldwide production and cultivated area of triticale have been steadily increasing (Salmon et al., 2004).

The area dedicated to growing canola is only exceeded by wheat production in Canada (FAO, 2011). The average cultivated area for canola in Canada over the last 10 years (1999–2009) has been 4.9 million ha (FAO, 2011). Using an average canola straw yield of 3 ton/ha (Yousefi, 2009), the average production of canola straw in Canada is 14.7 million metric tons annually. If it is assumed that canola straw has the same soil and livestock requirements as the cereal straw, then 6.0 million metric tons annually would be available to industry for energy and chemical conversion.

The lignocellulosic biorefinery principle aims to convert lignocellulosic biomass into a wide range of products and energy. The first step in the process is the pretreatment to fractionate the biomass into its main constituents, cellulose (30–50%), hemicellulose (15–35%), lignin (10–30%), and other minor components (Buranov and Mazza, 2008; da Costa Sousa et al., 2009; Tamaki and Mazza, 2010). Fractionation of lignocellulosics may be accomplished using various physical, biological, thermal, or chemical methods, although thermochemical fractionation is more suitable for commercial application at this time due to shorter processing times, higher yields, limited chemical use, and lower energy requirements (Kumar et al., 2009). There has been much research conducted into various thermochemical means for fractionating lignocellulosics including: steam explosion; ammonia fiber explosion (AFEX); dilute acid; alkaline; organosolv process; pressurized aqueous ethanol; pressurized aqueous ammonia treatments; ionic liquids; and hydrothermal treatment with pressurized low polarity water (PLPW) (Buranov and Mazza, 2010; da Costa Sousa et al., 2009;

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Fu et al., 2010; Kim and Lee, 2006; Mosier et al., 2005; Sun and Cheng, 2002). The benefits of hydrothermal treatment with PLPW over the other thermochemical methods include the low cost, reduced corrosion, lower concentrations of sugar degradation products, no generation of waste streams from neutralization of extracts, and the use of a non-toxic processing medium allows for the production of food grade products and pharmaceuticals.

Understanding the chemical composition of a specific lignocellulosic biomass before and after treatment is paramount if the success for its utilization within a biorefinery is to be ensured. There is considerable information on the effects of hydrothermal processing with PLPW on the fractionation and resulting product streams from many types of lignocellulosic biomass including: rapeseed (Díaz et al., 2010); rye straw (Gullón et al., 2010); corn stover (Liu and Wyman, 2003); sugarcane bagasse (Jacobsen and Wyman, 2002); almond shells (Nabarlatz et al., 2005); wheat straw (Petersen et al., 2009); triticale straw (Pronyk and Mazza, 2011); and flax shives (Buranov and Mazza, 2010). However, composition of the product streams is dependent on the starting material, and the design and operation of the chosen reactor system. As such, comparison of results from different lignocellulosic materials for different reactor systems is problematic.

Several researchers have compared the effects of hydrothermal processing of several different types of lignocellulosic biomass species under the same processing conditions. Parajó et al. (2004) compared the batch processing of *Eucalyptus globulus* wood, corn cobs, rice hulls, and barley hulls. The production of xylo-oligosaccharides from the biomass was not obviously correlated to starting composition of the material. Kabel et al. (2002a) studied the xylo-oligosaccharides produced from batch processing of *E. globulus* wood, corn cobs, brewery's spent grain, and wheat hulls. Hydrothermal treatment produced xylans with different molecular weights and structures. Xylans from brewery's spent grain contained arabinose substituents, whereas xylans from corn cobs and *Eucalyptus* wood were also substituted with 4-*O*-methylglucuronic acid. Mok and Antal (1992) looked at the hydrothermal processing of six woody and four herbaceous biomass species (*Eucalyptus gummifera*, *Eucalyptus saligna*, *Populus deltoids*, *Luecaena* hybrid KX3, silver maple, sweet gum, switch grass, sweet sorghum, sugar cane, and energy cane) in a small flow-through reactor (0.3 g sample, 4.6 mm ID × 7.6 cm length). They found that the extraction of cellulose and lignin was not correlated to the composition of the starting material. However, the total solubilized mass differed between biomass species and it was positively correlated to the starting hemicellulose content.

The purpose of this work was to evaluate the fractionation of five cereal (triticale, durum wheat, CPS wheat, feed barley, oats) and two oilseed (canola, mustard) straws using hydrothermal processing with pressurized low polarity water in a flow-through reactor. Mass balances and composition of the liquid extracts during the course of the hydrothermal processing of the straw with PLPW were quantified to determine the products that may be of interest within a lignocellulosic biorefinery.

2. Methods

2.1. Materials

Triticale (*Triticosecale* W.; cv. AC Ultima), durum wheat (*Triticum durum* L.), CPS wheat (*Triticum aestivum* L.), feed barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), canola (*Brassica napus* L.), and mustard (*Brassica carinata* A. Braun) straws were provided by the CBioN ABIP Network, Saskatoon, SK from Agriculture and Agri-Food Canada's Semiarid Prairie Agricultural Research Centre in Swift Current, SK. The straw (approximately 6% moisture content) was coarsely ground in a Retsch mill (Model SM 2000, Retsch GmbH, Haan, Germany) to pass through an 8 mm discharge screen. The ground sample was sieved for 5 min in a tapping sieve shaker (Model AS200Tap, Retsch GmbH, Haan, Germany) equipped with a No. 10 (2 mm size) and a No. 35 (0.5 mm size) sieve to separate out the long pieces of straw and fine particles. The ground sample was bagged and stored at −20 °C prior to use.

2.2. PLPW fractionation and extraction

Equipment used for the PLPW extractions was described by Pronyk and Mazza (2011). Pressure in the system was maintained at 11 MPa (1600 psi) for all experiments. Experiments for each straw were conducted twice with each run using 120 g of dry ground straw, uniformly packed into a stainless steel flanged column 50 mm ID × 500 mm length (MODcol, Mandel Scientific Company Inc., Guelph, ON) to a bed depth of 400 mm. To keep the sample inside the reaction vessel, and to help promote dispersion of the solvent, both ends were packed with 50 mm of stainless steel wool and capped with a 20 μm and 100 μm stainless steel frit at the inlet and outlet, respectively.

The optimum processing conditions for the hydrothermal treatment of triticale straw using PLPW, to achieve maximum yield of hemicellulose, was previously determined to be a temperature, flow rate, and solvent-to-solid ratio of 165 °C, 115 mL/min, and 60 mL/g respectively (Pronyk and Mazza, 2011). These operating conditions were used for the hydrothermal processing of all the straws used in these experiments.

The hydrothermal treatment procedure was initiated by first heating the filled reaction vessel while dry to the operating temperature, and holding it there for 1 h to equilibrate. The column was then flooded with PLPW before commencing flow through the system. The first portion of solution, which contained no extracted material (corresponding to the dead volume in the system from the top of the reaction vessel to the collection vessel), was discarded and the predetermined volume of solution (7.2 L total; 8 × 900 mL fractions) was collected based on the optimum solvent-to-solid ratio of 60 mL/g. Fractions 5 and 6, and fractions 7 and 8 were combined for compositional analysis to ensure there was enough material for all the tests. After each hydrolysis, the system was flushed with PLPW for 1 h to remove any residue. A portion (approximately 60 mL) of the liquid extracts was collected from each experiment were stored at −20 °C, the rest of the liquid extracts were freeze dried along with the solid residues and stored at −20 °C until they were analyzed.

2.3. Compositional analysis

2.3.1. Solid residues

Solid residues and freeze dried liquid extracts were analysed for structural carbohydrates, lignin, acetyl groups, and ash content according to NREL (National Renewable Energy Laboratory) standard analytical procedures (Hyman et al., 2007; Sluiter et al., 2008).

Acid insoluble lignin (AIL) and acid soluble lignin (ASL) were determined by first hydrolysing samples with 72% sulphuric acid for 1 h at 30 °C in a water bath and then diluting to 4% sulphuric acid and autoclaving at 121 °C for 1 h in sealed glass pressure tubes. AIL was analysed gravimetrically after the hydrolysis of the cellulose and hemicellulose (Sluiter et al., 2008). ASL in the hydrolysate was determined by the spectrophotometric method at 320 nm (Hyman et al., 2007). An absorptivity of 30 L g^{-1} cm^{-1} was used to convert absorbance readings to mass values. The results for lignin content of the samples are reported as the sum of the AIL and ASL and are corrected for protein content (Pronyk et al., 2011).

Structural carbohydrates, cellulose (glucose) and hemicellulose (xylose, galactose, arabinose, and mannose) were determined quantitatively from the hydrolysate by HPLC using an Agilent 1100 equipped with a refractive index detector (Agilent Technologies, Palo Alto, CA). The HPLC analysis was carried out using an Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad Laboratories, Hercules, CA) with a deashing guard cartridge (Bio-Rad Laboratories, Hercules, CA) operating at 75 °C. The HPLC system consisted of a G1329A autosampler and G1312A delivery system that were controlled by Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). HPLC grade filtered water (Milli-Q) was used as the mobile phase at a flow rate of 0.5 mL/min and, for each sample, 50 μL of prefiltered aliquot was injected automatically. The carbohydrate concentrations were determined by comparison against a set of known sugar standards and the application of a sugar recovery factor according to NREL standard procedures (Sluiter et al., 2008).

Acetyl groups, formic and levulinic acids were quantitatively measured from the hydrolysate with HPLC using an Agilent 1100 equipped with a refractive index detector (Agilent Technologies, Palo Alto, CA) (Sluiter et al., 2008). The HPLC analysis was conducted using a Bio-rad Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Hercules, Ca) with a Cation H refill Cartridge guard column (30 × 4.6 mm, Bio-Rad Laboratories, Hercules, CA) operating at 55 °C with a 0.005 M H_2SO_4 mobile-phase at a flow rate of 0.6 mL/min.

Uronic acids in the hydrolysate were quantified by the Scott method (Scott, 2002). An aliquot (0.125 mL) of the hydrolysate was added to 0.125 mL of 2% NaCl-3% H_3BO_3 solution in a test tube. Concentrated H_2SO_4 was added to the test tube in an ice bath and mixed. The test tube was then heated for 40 min at 70 °C in a water bath. The test tubes were then removed and allowed to cool to room temperature before 0.1 mL of 0.1% 3,5-dimethylphenol in glacial acetic acid was added to the reactant. After 10 min, the uronic acids concentration was determined by averaging the absorbance at 400 and 450 nm and comparing it to a standard curve of D-glucuronic acid (Sigma–Aldrich Co., St. Louis, MO).

Ash content of the solids was determined by complete combustion of the samples in a muffle furnace (Model F-A1730, Thermolyne Corporation, Dubuque, IA) equipped with a temperature controller (Furnatrol II series 413, Thermolyne Corporation, Dubuque, IA). The temperature controller was set to ramp up to 105 °C from room temperature, held for 12 min, ramped up to 250 °C at 10 °C/min, held for 30 min, ramped up to 575 °C at 20 °C/min, held for 180 min, and dropped to 105 °C and held until the sample was removed. The remaining residue in the crucible was taken as the ash content.

Protein content was estimated from the nitrogen content of the samples (AOAC, 2008). Prior to analysis the solid residues were ground in a hammer mill (MF 10, IKA-Werke GmbH & Co. KG, Staufen, Germany) to pass through a 0.5 mm discharge screen. Samples were dried overnight in a vacuum oven at 60 °C prior to analysis. Nitrogen content was determined by combusting the dried samples at 850 °C using a Leco FP-528 nitrogen analyser (Leco Corporation, St. Joseph, MI). A standard curve for nitrogen was produced using ethylenediaminetetraacetic acid (EDTA) and corn flour (Leco Corporation, St. Joseph, MI). Protein content was estimated by multiplying the nitrogen content (%) by a factor of 6.25.

Lipid content of the native straw samples was determined according to the procedure described by Athukorala and Mazza (2010) with minor modifications. Ground straw samples (~3 g) were extracted thoroughly with hexane (45 mL) (purchased from Sigma–Aldrich, Oakville, ON) in a Goldfisch fat extraction apparatus (Labconco, Kansas City, MO) for 6 h. Lipids were collected after the solvent was evaporated in a vacuum oven and the lipid content was determined gravimetrically.

2.3.2. Liquid extracts

Liquid extracts were neutralised with calcium carbonate, filtered through a 0.20 μm syringe filter, and used for direct HPLC determination of carbohydrate monomers. The concentration of carbohydrate oligomers was then calculated by taking the difference between the hydrolysed total carbohydrate content determined from the freeze dried extracts and the monomer content determined from the liquid samples. The degradation products 5-hydroxy-2-methylfurfural (HMF) and furfural were determined from the same sample by direct HPLC determination using DAD detection.

2.4. Statistical analysis

Data were analysed using the ANOVA procedure of SAS (2009) (v9.2, SAS Institute Inc., Cary, NC) and a means comparison by Tukey's test was performed. Differences with $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Native straw composition

The various samples were first analyzed to determine the initial quantity of each component available in the native straw material (Table 1). Compositional analysis was performed using native straw material, not material extracted with water and ethanol to remove the extractives as specified by the NREL laboratory procedure, except for lignin content, which was determined using the extractives free material (Sluiter et al., 2008). This was done because industry would use the material as received, which still contains the extractives. These extractive materials can have a significant effect on the measured lignin and ash contents, thus lignin contents were determined using extractive free material. However, there is no effect on the glucan and xylan content of triticale straw with the removal of the extractives (Tamaki and Mazza, 2010) and subsequently the use of native and not extractive free straw should still produce accurate results.

Composition varied between the different crop species, especially between the cereal and oilseed crops (Table 1). The oilseed crops, canola and mustard, had in general lower quantities of the structural carbohydrates glucan, xylan, and arabinan but contained larger amounts of galactan and mannan. The main xylan backbone of the oilseed straw contained more uronic and acetyl substituents than the cereal straw. The content of acetyl groups in the oilseed crops was nearly twice that of the cereal crops and for uronic acids it was more than four times greater. The presence of acetyl and uronic substiuents linked to xylo-oligosaccharides may impart unique benefits and produce applications in the manufacture of prebiotic food ingredients (Parajó et al., 2004). In addition, acetic acid produced from acetyl groups and released uronic acids may aid in the hydrolysis of hemicellulose with hydrothermal treatment. Triticale had the highest xylan content and the lowest lignin content of all the straws. This is potentially beneficial for the hydrothermal process because lignin does not exist as an independent polymer in plant tissue, but is bonded with cellulose and hemicellulose (Buranov and Mazza, 2008). The lower lignin content of triticale could make the hemicellulose more accessible to hydrolysis, resulting in higher yields. Alternatively, triticale has the highest ash content due to the large amount of extractive material it contains (Tamaki and Mazza, 2010). Ash may have neutralizing ability which may impact the effectiveness of the hydrothermal treatment (Parajó et al., 2004). There is a wide variation in protein content amongst the straw material, with canola containing the lowest at 2.17%, and CPS wheat containing the most at 5.10% (Table 1). The

Table 1
Composition of starting material.

Constituents (%: dry weight basis)	Straw material^{A}						
	Triticale	Durum wheat	CPS wheat	Barley	Oats	Canola	Mustard
Glucan	36.28^{c}	39.42^{a}	37.88^{b}	40.10^{a}	39.81^{a}	32.49^{e}	33.40^{d}
Xylan	21.01^{a}	19.68^{b}	19.30^{bc}	18.98^{c}	19.95^{b}	15.65^{d}	14.68^{e}
Galactan	1.21^{b}	0.86^{d}	1.11^{bc}	0.98^{cd}	1.08b^{c}	1.97^{a}	2.05^{a}
Arabinan	2.12^{a}	1.64^{d}	1.82^{c}	1.93^{bc}	1.99^{ab}	0.76^{f}	1.23^{e}
Mannan	0.42^{d}	0.32^{d}	0.81^{c}	0.30^{d}	0.40^{d}	2.24^{a}	1.69^{b}
Lignin^{B}	16.12^{c}	18.15^{b}	18.38^{b}	19.37^{a}	18.20^{b}	19.28^{a}	18.38^{b}
Acetyl groups	2.32^{c}	2.34^{c}	2.43^{c}	1.68^{d}	1.82^{d}	4.32^{a}	3.75^{b}
Uronic acid	1.44^{c}	1.59^{c}	1.57^{c}	1.82^{c}	1.67^{c}	6.85^{b}	8.21^{a}
Protein	3.82^{b}	2.37^{e}	5.10^{a}	3.38^{c}	2.49^{d}	2.17^{f}	2.55^{d}
Lipids	0.84^{a}	0.49^{b}	0.57^{b}	0.79^{a}	0.92^{a}	0.83^{a}	0.76^{a}
Ash	7.51^{a}	4.54^{b}	4.45^{b}	3.47^{d}	3.92^{c}	3.16^{e}	3.34^{d}
Others (by difference)	6.91	8.60	6.58	7.20	7.75	10.28	9.96

[A] Mean values in a row with different superscript letters are significantly different ($p < 0.05$).

[B] Extractives free.

Table 2
Mass balance of hydrothermal processing with PLPW.

	Straw material^{A}						
	Triticale	Durum wheat	CPS wheat	Barley	Oats	Canola	Mustard
Starting material (g)	120.00	120.00	120.00	120.00	120.00	120.00	120.00
Solid residue (g)	57.30^{c}	63.08^{a}	59.12^{bc}	61.80^{ab}	61.82^{ab}	57.62^{c}	60.06^{abc}
Dissolved mass (g)	55.72^{a}	52.38^{ab}	55.68^{a}	52.89^{ab}	53.72	53.42^{ab}	50.96^{b}
Total (g)	113.02	115.46	114.80	114.69	115.54	111.04	111.02
Unaccounted Material (losses)^{B} (g)	6.98	4.54	5.20	5.31	4.46	8.96	8.98

[A] Mean values in a row with different superscript letters are significantly different ($p < 0.05$).

[B] Calculated as Starting material – Solid residue – Dissolved mass.

lipid content, which includes waxes and other lipophilic compounds, was low for all straws and only the two wheat straws were statistically different ($p < 0.05$).

3.2. Material balance

The mass balance after hydrothermal processing was in good agreement for all straw material (Table 2). There was only a small variation in dissolved mass for all the straws, with mustard the lowest at 50.96 g (42.5% of the starting mass) and triticale the highest at 55.72 g (46.4% of the starting mass), suggesting that there is a weak correlation between dissolved mass and hemicellulose content (Table 1), as found by other researchers (Mok and Antal, 1992). Barley, which had the highest lignin content of the cereal straws, produced the lowest dissolved mass from the hydrothermal treatment. Conversely, triticale with the lowest lignin content, achieved the highest dissolved mass of all the straws from the hydrothermal treatment. The oilseeds, which contained the greatest amount of substituents for producing in situ acids, may be expected to be more readily hydrolysed during the treatment, but canola only achieved the same dissolved mass as most of the cereals and mustard achieved the lowest of all the straw species. Thus results indicate that the greater acetyl and uronic acids content of the oilseeds did not influence the hydrolysis and that lignin content of the straw plays a more important role.

The amounts of solid residues remaining after hydrothermal treatment were similar for all the straws and variation is partly due to losses which occurred during processing. These unaccounted losses were greatest for the oilseed straws; where close to 9 g (7.5%) of the staring material was lost. Most of the losses occurred from the hemicellulose fraction of the straw, including the acetyl groups and uronic acids. Hemicellulose is more susceptible to thermal degradation than the cellulose and lignin fractions, whereby the hemicellulose pentose and hexose carbohydrates are converted to the degradation products furfural and HMF respectively. A portion of the hemicellulose fraction might have undergone some form of pyrolysis or torrefaction during the hydrothermal treatment, resulting in a portion of the carbohydrates converting to gaseous form. These losses due to pyrolysis are not likely to be large, only 3% of solid waste was converted to gaseous products at 230 °C (Demirbaş and Arin, 2002), but it would account for some of the lost material. Large amounts of the uronic acids are unaccounted for in the mass balance, with losses of nearly 50% in the cereal straws and over 75% in the oilseed straws. Uronic acids thermally degrade to 2-furancarboxylic and 5-formyl-2-furancarboxylic acids (Li et al., 2007), which were not measured in these experiments. Acetyl group losses were on the order of 20–40% for triticale, canola, and mustard. Acetic acid generated from the acetyl groups during hydrothermal processing would be evaporated from the liquid samples during freeze drying, and thus would be lost from the mass balance along with other volatile compounds.

3.3. Composition of the solid residue and liquid fractions

3.3.1. Carbohydrates

Compositions of the solid and liquid fractions from the PLPW extraction of the straw samples are presented in Table 3. Glucan (cellulose) content of all straw samples was enriched by the hydrothermal process. There was little difference between straw samples except for canola, mustard, and triticale which had 2–6% less glucan. This is consistent with the lower initial glucan content of these straws (Table 1). Yield of glucose in the liquid fractions was low and represented less than 10% of the available glucose (Fig. 1a). Glucan is not readily hydrolysed because of its configuration, which promotes the ordering of the polymer chains into tightly packed, highly crystalline structures. Previous research has suggested that most of the glucose from flow-through hydro-

Table 3
Composition of solid residues and freeze dried liquid extracts.

Constituents (%: dry weight basis)	Straw material[A]						
	Triticale	Durum wheat	CPS wheat	Barley	Oats	Canola	Mustard
Solid residue							
Glucan	65.99^{b}	68.72^{ab}	68.25^{ab}	67.90^{ab}	70.99^{a}	64.18^{b}	66.16^{b}
Xylan	4.24^{a}	3.56^{a}	3.25^{a}	3.71^{a}	3.63^{a}	3.82^{a}	4.69^{a}
Galactan	0.60^{ab}	0.41^{ab}	0.52^{ab}	0.33^{b}	0.53^{ab}	0.54^{ab}	0.63^{a}
Arabinan	0.18^{ab}	0.30^{ab}	0.06^{b}	0.41^{a}	0.18^{ab}	0.08^{b}	0.20^{ab}
Mannan	0.14^{a}	0.27^{a}	0.36^{a}	0.26^{a}	0.52^{a}	1.11^{b}	1.50^{b}
Lignin[B]	18.12^{b}	18.70^{b}	17.36^{b}	18.49^{b}	18.66^{b}	22.92^{a}	22.50^{a}
Acetyl groups	0.41^{a}	0.48^{a}	0.62^{a}	0.37^{a}	0.36^{a}	0.63^{a}	0.69^{a}
Uronic acid	0.38^{ab}	0.26^{b}	0.55^{ab}	0.24^{b}	0.28^{b}	0.36^{ab}	0.64^{a}
Protein	2.48^{a}	1.42^{bc}	2.74^{a}	1.87^{b}	1.36^{c}	1.48^{bc}	1.88^{b}
Ash	3.71^{a}	1.19^{b}	1.66^{b}	1.27^{b}	1.61^{b}	0.36^{c}	0.11^{c}
Others (by difference)	3.75	4.69	4.63	5.15	1.88	4.52	1.00
Liquid fractions (*dissolved mass*)							
Gluco-oligosaccharides (GO)	4.83^{c}	5.04^{c}	7.72^{a}	6.32^{b}	5.10^{c}	3.44^{d}	2.33^{e}
Xylo-oligosaccharides (XO)	30.83^{ab}	33.90^{a}	31.64^{a}	32.28^{a}	30.54^{ab}	26.85^{b}	26.69^{b}
Galacto-oligosaccharides (GOS)	1.07^{a}	1.06^{a}	1.17^{a}	1.04^{a}	1.42^{a}	2.26^{a}	2.12^{a}
Arabino-oligosaccharides (ArO)	1.48^{ab}	1.04^{abc}	1.20^{abc}	1.74^{a}	1.50^{ab}	0.78^{bc}	0.40^{c}
Manno-oligosaccharides (MOS)	0.74^{c}	0.32^{c}	1.44^{bc}	0.49^{c}	0.50^{c}	2.73^{a}	2.64^{ab}
Glucose	0.45^{a}	0.56^{a}	0.51^{a}	0.55^{a}	0.52^{a}	0.74^{a}	0.64^{a}
Xylose	1.90^{b}	3.98^{a}	2.26^{ab}	2.58^{ab}	2.30^{ab}	1.70^{b}	1.80^{b}
Galactose	1.04^{a}	0.99^{a}	0.54^{b}	0.97^{ab}	1.04^{a}	1.00^{a}	0.82^{ab}
Arabinose	1.42^{ab}	1.74^{a}	1.60^{a}	1.97^{a}	1.70^{a}	0.86^{bc}	0.81^{c}
Mannose	0.31^{b}	0.42^{ab}	0.39^{ab}	0.34^{b}	0.42^{ab}	0.68^{a}	0.56^{ab}
HMF	0.04^{a}	0.07^{a}	0.08^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.07^{a}
Furfural	0.51^{a}	0.80^{a}	0.70^{a}	0.50^{a}	0.60^{a}	0.48^{a}	0.52^{a}
Lignin[B]	17.34^{c}	22.76^{ab}	13.40^{d}	20.11^{bc}	23.18^{ab}	22.25^{ab}	24.94^{a}
Acetyl groups	2.62^{d}	4.06^{b}	3.81^{bc}	3.14^{cd}	2.91^{d}	5.97^{a}	6.16^{a}
Uronic acid	1.21^{c}	1.27^{c}	1.11^{c}	1.72^{abc}	1.66^{bc}	3.24^{ab}	3.57^{a}
Formic acid	0.96^{bc}	0.65^{c}	0.87^{bc}	0.72^{c}	0.58^{c}	1.79^{a}	1.31^{ab}
Levulinic acid	0.21^{a}	0.16^{a}	0.34^{a}	0.32^{a}	0.33^{a}	0.29^{a}	0.24^{a}
Protein	5.82^{b}	3.74^{de}	7.66^{a}	5.15^{bc}	3.75^{de}	3.65^{e}	4.57^{cd}
Ash	13.06^{a}	6.28^{c}	7.88^{b}	6.13^{c}	5.79^{c}	6.77^{bc}	5.67^{c}
Others (by difference)	14.17	11.15	15.69	13.88	16.10	14.47	14.14

[A] Mean values in a row with different superscript letters are significantly different ($p < 0.05$).
[B] Corrected for protein.

thermal treatment is probably associated with the hemicellulose or extractives, and not with the cellulose fraction of the straw (Pronyk et al., 2011). There was a minor amount of glucose monomers in the liquid extracts and there was no difference between straws ($p > 0.05$). There was significant variation in gluco-oligosaccharides between straws ($p < 0.05$), with the oilseed straws producing less than the cereal straws.

There was no difference in xylan content of the residues ($p > 0.05$) and the amount of xylan remaining was low, indicating a large degree of hemicellulose hydrolysis occurred during the hydrothermal treatment (Table 3). The remaining xylan in the solid residue samples is probably more tightly bound to the lignin, making it unsusceptible to hydrolysis under the processing conditions used in this study (Conner and Lorenz, 1986; Garrote et al., 2001). Recovery of xylose in the liquid fractions was high for all crops (Fig. 1a). Canola and mustard yielded almost 10% (25 g/kg dry straw) less xylose than the cereal straw, but this was probably due to the lower xylan content of the original straw. Hydrolysed xylan was recovered in the liquid extracts mostly in oligomer form (Table 3). Xylo-oligosaccharide concentration from the oilseed straw may be lower, but they are likely to contain more uronic and acetyl substituents than the cereal straw due to the higher yield of uronic acids and acetyl groups (Fig. 1b). Substituted xylo-oligosaccharides such as those obtained from mustard and canola could be of great interest for their potential use in pharmaceutical and food applications (Moure et al., 2006; Kabel et al., 2002b).

Concentrations of the minor carbohydrates galactan, arabinan, and mannan in the solid residues were low, except for mustard and canola residues, which contained more mannan (Table 3). Dicot species such as canola and mustard contain more mannans and glucomannans than monocot species such as grass (Vogel, 2008), hence the greater mannan in the solid residues of the oilseed straw. The hemicellulose in the oilseeds is a xylo glucan, whereas in the cereals it is a glucuronoarabinoxylan (Vogel, 2008). Thus arabinan yield was greatest for the cereal straws, whereas oilseed straws yielded more galactan and mannan (Fig. 1a). Overall yields of galactan, arabinan, and mannan in the liquid extracts were low and yields of less than 14 g/kg of straw were achieved. For the cereal straws arabinan was hydrolysed easily into its monomer, arabinose. For canola and mustard the concentrations of mannose was almost as large as the concentration of glucose, and they were mostly obtained as oligosaccharides (Table 3).

3.3.2. Lignin

Lignin content for the solid residues and liquid fractions has been corrected for protein. Samples are corrected for protein because protein is largely acid insoluble and must be taken into account in the gravimetric determination of lignin to avoid an overestimation of the true lignin content (Sluiter et al., 2008; Hatfield and Fukushima, 2005). There was no difference in lignin content for the cereal straw residues ($p > 0.05$). Lignin content of the oilseed straw residues was more than 4% higher than the cereal straw residues. This could have implications for the production of ethanol because the residues would have to undergo secondary enzymatic hydrolysis to produce glucose monosaccharides for fermentation. Lignin is known to restrict enzymatic hydrolysis by adsorbing and inactivating enzymes (Wyman et al., 2005). Therefore, increased lignin contents for canola and mustard could reduce glucose yields from the residues by enzymatic hydrolysis and

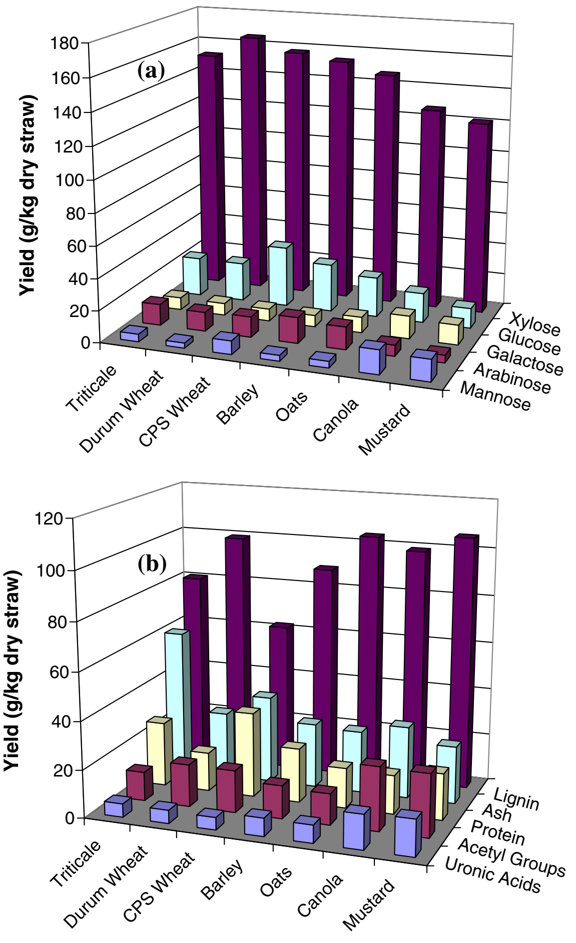

Fig. 1. Product yield obtained in freeze-dried liquid fractions from different straw species after hydrothermal treatment: (a) carbohydrates (monomers and oligosaccharides), and (b) non-carbohydrate products.

thereby affect the potential ethanol yield. Freeze dried extracts from canola and mustard straw contained the same amount of lignin ($p > 0.05$) as the durum wheat and oat straws (Table 3). Triticale and barley straws contained intermediate amounts of lignin and CPS wheat extracts contained the least lignin. CPS wheat yielded 20–40% less lignin than the other straws (Fig. 1b).

3.3.3. Minor components

The content of minor components in the solid residues was similar for all straws, but there was more variability in the freeze dried liquid extracts (Table 3). In the residues, concentrations of acetyl groups were the same ($p > 0.05$) and uronic acids were only slightly higher for the mustard straw. Canola and mustard extracts contained more acetyl groups and uronic acids than the cereal extracts; in most cases they contained almost twice as much as the cereal straws.

Concentration of protein in the residues and freeze dried extracts was correlated to initial content of protein in the starting materials. CPS wheat straw, which had the largest initial of protein content, produced a residue and freeze dried extract with the greatest concentration of protein. However the differences in protein concentration in residues and extracts after hydrothermal treatment were much less than within the starting materials (Table 3). Deviation in protein content of the residues was small and amounted to less than 1.5 g of the residue mass. However, there was a much larger deviation in protein content of the extracts, but the amount obtained was still only 2.0–4 g in the freeze dried material.

Ash in the straw was readily extractable during hydrothermal processing and yielded the third highest amount of material in the extracts for all of the straws after xylose and lignin (Fig. 1b). Triticale straw had the highest initial ash content of all the straws and in return after hydrothermal treatment the residue and freeze dried liquid extracts also contained the largest amount of ash of all the straws processed. The other cereal straw residues, even with variable initial ash contents, contained the same concentration of ash after hydrothermal treatment ($p > 0.05$). The ash in oilseed

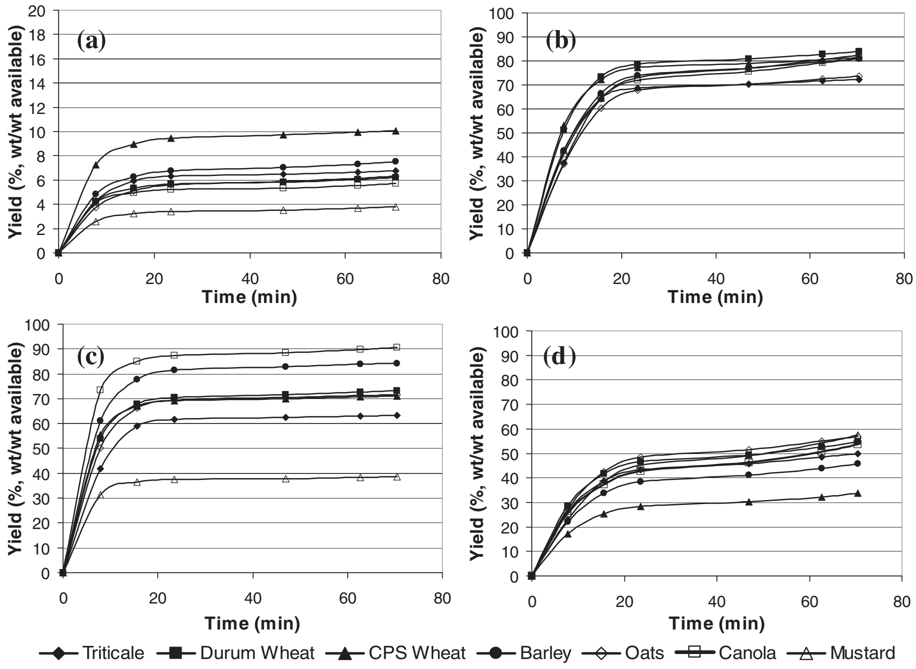

Fig. 2. Major product recovery from the reactor during flow-through hydrothermal treatment of different crop species: (a) glucose, (b) xylose, (c) arabinose, and (d) lignin.

straws turned out to be readily extractible and the resulting residues contained significantly less ash than the other straws, yielding a residue with only 0.11–0.36% ash. Lower residue ash content from hydrothermal pretreatment of straw is a benefit during ethanol production. In a biorefinery, after fermentation of the carbohydrates in the pretreated straw, a lignin rich waste material is obtained. This material is often combusted as a boiler fuel and ash is the waste product that results, which must be disposed of at additional cost. By utilizing low ash PLPW pretreated canola or mustard straw residues, ethanol production costs could be reduced by lowering waste disposal costs.

3.3.4. Extraction of products

The yield of glucose, xylose, arabinose, and lignin in the liquid extracts with processing time are plotted in Fig. 2. These products represent the compounds with the greatest yielded mass, and they would be of most interest within a biorefinery. The reaction and extraction of these products from the reactor system during hydrothermal treatment was largely completed within the first 20–30 min. Small increases in yield are achieved after this time, but it comes at the expense of lower product concentrations. This in turn would add to the cost of processing the extracts into usable products.

The kinetics of the xylose hydrolysis is largely independent of the starting material (Fig. 2b). Regardless of starting material, the flow-through hydrothermal treatment was successful in yielding 70–80% of the original xylose contained in the straw. When examining the remaining xylan in the residue after hydrothermal treatment it is seen that 90% or greater of the starting xylose was removed. The difference between the remaining and yielded xylose was due to the production of degradation products.

The yield of glucose and arabinose during hydrothermal treatment was dependent on the starting material (Figs. 2a and c). In addition there seems to be no correlation between starting concentrations and final yields of these two compounds. It is likely that structure of the carbohydrates within the native straw material (glucuronoarabinoxylan hemicellulose in the cereal straw compared to the xyloglucan of the oilseeds) plays an important role in the yield of these compounds. Arabinose yield was the highest for all the main products due to the high susceptibility of arabinose to hydrolyse during hydrothermal treatment (Pronyk et al., 2011).

Similar to results for xylose, lignin yield was mostly independent of the starting material (Fig. 2d). The exception was for CPS wheat straw, which yielded much lower amounts of lignin when compared to the other crops. Lignin does not exist in plant tissue as an independent polymer, but is bonded with cellulose and hemicellulose, forming complexes with them (Buranov and Mazza, 2008). Hence the extraction of xylose would be expected to depend on the extraction of the lignin. In these experiments the ratio of lignin to xylose extraction ranged from 0.4 to 0.88, with CPS wheat the lowest and mustard straw the highest.

4. Conclusions

Hydrothermal processing with PLPW was successful in fractionating all biomass species, leaving a cellulose rich solid residue and producing an extract rich in oligo-saccharides. Cereal straws yielded a residue richer in cellulose and lower in lignin. Oilseed straws yielded a residue lower in ash. Xylo-oligosaccharides potentially contain more acetyl and uronic substituents, making them of higher value for use in food applications as prebiotic compounds. Yields of xylose and lignin were not dependent on the starting material but the total quantity of products was often correlated to the starting composition.

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